4.0 *IN VITRO* SKIN TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

4.1 Background

Prevalidation and validation studies have been completed for the rat skin TER assay (ICCVAM 2002; Fentem et al. 1998; Oliver et al. 1986; Oliver et al. 1988; Botham et al. 1992; Botham et al. 1995; Barratt et al. 1998). Based on its scientific validity, this test method has been recommended for the testing of all classes of chemicals (ICCVAM 2002; Fentem et al. 1998; Balls and Corcelle 1998a) and for inclusion in tiered testing strategies as part of a tiered or weight-of-evidence evaluation (ICCVAM 2002). This chapter briefly describes the principles of the *in vitro* skin TER test for corrosivity followed by the recommended performance standards, which consists of essential test method components, reference chemicals, and comparison of accuracy and reliability.

4.2 Principles of the *In Vitro* Skin TER Test for Skin Corrosion

The test material is applied for up to 24 hours to the epidermal surfaces of skin discs in a two compartment test system in which the skin discs function as the separation between the compartments. The skin discs are prepared from humanely killed 28-30 day old rats. Corrosive materials are identified by their ability to produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction in the TER below a threshold level (Oliver et al. 1986). For rat skin TER, a cutoff value of 5 kΩ has been selected based on extensive data for a wide range of chemicals where the vast majority of values were either clearly well above or well below this value (Oliver et al. 1986). Generally, chemicals that are noncorrosive but irritating in animals do not reduce the TER below this cutoff value. However, the use of other skin preparations or other equipment may alter the cutoff value, necessitating further validation. A dye-binding step is incorporated into the test procedure for confirmation testing of positive results in the TER. The dye-binding step determines if the increase in ionic permeability is due to physical destruction of the stratum corneum.

Investigators using an *in vitro* skin TER corrosivity test must be able to demonstrate that the assay is valid for its intended use. This includes demonstrating that different preparations are consistent in barrier properties (i.e., capable of maintaining a barrier to noncorrosive substances, able to respond appropriately to weak and strong corrosive substances) and/or that any modification to the existing validated reference test method does not adversely affect its performance characteristics.

The *in vitro* TER test for skin corrosion may be used to test solids, liquids, and emulsions of any chemical or product class. The liquids can be aqueous or nonaqueous; solids can be soluble or insoluble in water. The samples may be pure chemicals, dilutions, formulations, or waste. Where appropriate, solids can be heated to 300°C to melt or soften the test material or ground to a powder before application; no other prior treatment of the sample is required. In some chemical classes, relatively few chemicals were included in the validation of the accepted *in vitro* rat skin TER test for skin corrosion (Fentem et al. 1998). However, considering the limited mechanisms that result in corrosivity, this method is expected to be generally applicable across all chemical classes (ICCVAM 2002; Fentem et al. 1998; Balls and Corcelle 1998a).

4.3 Essential Test Method Components

The following is a description of the essential test method components of the *in vitro* skin TER test for skin corrosivity, as provided in the OECD Test Guideline 430 (OECD 2003b).

4.3.1 Animals

All procedures involving the use of animals should be in compliance with relevant national animal welfare act regulations and policies, and the studies should be approved by the Institutional Animal Care and Use Committee or its equivalent. Rats are the species of choice because the sensitivity of their skin to chemicals in this test has been previously demonstrated (Oliver et al. 1986). The age (when the skin is collected) and strain of the rat is particularly important to ensure that the hair follicles are in the dormant phase before adult hair growth begins. The use of skin from another species is possible as long as the test system is appropriately calibrated and the reliability and accuracy, using at the minimum, the provided list of reference chemicals (**Table 4-3**), is determined to be at least comparable to the performance characteristics of the validated reference test method.

If rat skin is used, the dorsal and flank hair from young, approximately 22 day-old, male or female rats (Wistar-derived or a comparable strain), is carefully removed with small clippers. Then, the animals are washed by careful wiping, while submerging the clipped area in antibiotic solution (containing, for example, streptomycin, penicillin, chloramphenicol, and amphotericin, at concentrations effective in inhibiting bacterial growth). Animals are washed with antibiotics again on the third or fourth day after the first wash and are used within three days of the second wash, when the stratum corneum has recovered from the hair removal.

4.3.2 Preparation of Skin Discs

Animals are humanely killed when 28-30 days old; this age is critical to the performance of the assay. The dorsolateral skin of each animal is then removed and stripped of excess subcutaneous fat by carefully peeling it away from the skin. Skin discs, with a diameter of approximately 20 mm each, are excised. The skin may be stored prior to use provided that positive and negative control data are equivalent to that obtained with fresh skin.

Each skin disc is placed over one of the ends of a polytetrafluoroethylene (PTFE) tube, ensuring that the epidermal surface is in contact with the tube. A rubber 'O' ring is press-fitted over the end of the tube to hold the skin in place and excess tissue is trimmed away. Tube and 'O' ring dimensions are provided in OECD Test Guideline (OECD 2003b). The rubber 'O' ring is then carefully sealed to the end of the PTFE tube with petroleum jelly. The tube is supported by a spring clip inside a receptor chamber containing $MgSO_4$ solution (154 mM) (OECD 2003b). The skin disc should be fully submerged in the $MgSO_4$ solution. As many as 10-15 skin discs can be obtained from a single rat skin.

Before testing begins, the electrical resistance of two skin discs is measured as a quality control procedure for each animal skin pelt. If both discs have resistance values greater than 10 kΩ then the remainder of the discs may be used for the test. If the resistance value is less than 10 kΩ, the remaining discs from that skin pelt should be discarded.

4.3.3 Application of Test Substances

Liquid test substances (150 µL) are applied uniformly to the epidermal surface inside the tube. When testing solid materials, a sufficient amount of the solid is applied evenly to the disc to ensure that the whole surface of the epidermis is covered. In order to achieve maximum contact with the skin, solids may need to be warmed to 300°C to melt or soften the test substance, or ground to produce a granular material or powder. Deionized water (150 µL) is added on top of the solid and the tube is gently agitated.

Three skin discs are used for each test and control substance; skin discs from a single animal should be used. Test substances are applied for 24 hours at 20-23°C. The test substance is removed by washing with a jet of tap water at temperatures up to 30°C, until no further material can be removed.

4.3.4 Control Substances

Solvent Controls: In tests that involve the use of a vehicle or solvent with the test substance, the vehicle or solvent must be compatible with the barrier system (i.e., not alter the integrity of the membrane barrier system) and should not alter the corrosivity of the test substance. When applicable, solvent (or vehicle) controls should be tested concurrently with the test substance to demonstrate the compatibility of the solvent with the barrier system.

Positive (Corrosive) Controls: A positive control chemical should be tested concurrently with the test substance to demonstrate that the *in vitro* skin TER test method is functioning properly. The positive control should be well-characterized for its corrosive activity and should generate a resistance value that is low to intermediate within the range of corrosive responses for this assay. An acceptable positive control response range must be developed based on historical positive control(s) data. In each test, the positive control should be evaluated to determine if the value is within the acceptable positive control range. Typically, for biologically-based test methods, acceptable ranges are within 2 to 3 standard deviations of the historical mean response but tighter ranges may be established by the developer of a proprietary test. 10 M Hydrochloric acid is an example of a positive control substance used in the rat skin TER assay.

Negative (Noncorrosive) Controls: A noncorrosive substance should also be tested concurrently with the test substance as another quality control measure to demonstrate the functional integrity of the human skin membrane barrier. An examples of a noncorrosive substance used as a negative control in the validated reference test method is distilled water.

Benchmark Controls: Benchmark controls may be useful to demonstrate that the test method is functioning properly for detecting the dermal corrosivity potential of chemicals of a specific chemical class or a specific range of responses, or for evaluating the relative corrosivity potential of a corrosive test substance. Appropriate benchmark controls should have the following properties:

- consistent and reliable source(s) for the chemical
- structural and functional similarity to the class of the substance being tested
- known physical/chemical characteristics
- supporting data on known effects in animal models
- known potency in the range of response (including moderate response)

4.3.5 TER Measurements

The skin impedance is measured as TER by using a low-voltage, alternating current Wheatstone bridge (Oliver et al. 1986). General specifications of the bridge are 1-3 V operating voltage, a sinus or rectangular shaped alternating current of 50–1000 Hz, and a measuring range of at least 0.1 -30 kΩ. For the TER corrosivity assay, measurements are recorded in resistance, at a frequency of 100 Hz and using series values. Prior to measuring the electrical resistance, the surface tension of the skin is reduced by adding a sufficient volume of 70% ethanol to cover the epidermis. After a few seconds, the ethanol is removed from the tube and the tissue is then hydrated by the addition of 3 mL $MgSO_4$ solution (154 mM). The databridge electrodes are placed on either side of the skin disc to measure the resistance in kΩ/skin disc (OECD 2003b). Electrode dimensions and the length of the electrode exposed below the crocodile clips are provided in the OECD Test Guideline (OECD 2003b). The clip attached to the inner electrode is rested on the top of the PTFE tube during resistance measurement to ensure that a consistent length of electrode is submerged in the $MgSO_4$ solution. The outer electrode is positioned inside the receptor chamber so that it rests on the bottom of the chamber. The distance between the spring clip and the bottom of the PTFE tube is maintained as a constant (Balls and Corcelle 1998a), because this distance affects the resistance value obtained. Consequently, the distance between the inner electrode and the skin disc should be constant and minimal (1-2 mm).

If the measured resistance value is greater than 20 kΩ, this may be due to the remains of the test substance coating the epidermal surface of the skin disc. Further removal of this coating can be attempted, for example, by sealing the PTFE tube with a gloved thumb and shaking it for approximately 10 seconds; the $MgSO_4$ solution is discarded and the resistance measurement is repeated with fresh $MgSO_4$.

The properties and dimensions of the test apparatus and the experimental procedure used may influence the TER values obtained. The 5 kΩ corrosive threshold was developed from data obtained with the specific apparatus and procedure described by OECD in Test Guideline 430. Different threshold and control values may apply if the test conditions are altered or a different apparatus is used. Therefore, it is necessary to calibrate the methodology and resistance threshold values by testing a series of calibration chemicals (see Section 4.4).

4.3.6 Dye-Binding Methods

Exposure of certain noncorrosive materials can result in a reduction of resistance below the cutoff of 5 kΩ allowing the passage of ions through the stratum corneum, thereby reducing the electrical resistance (Fentem et al. 1998). For example, neutral organics and chemicals that have surface-active properties (including detergents, emulsifiers, and other surfactants) can remove skin lipids making the barrier more permeable to ions. Thus, if the rat skin TER values of test substances are less than or around 5 kΩ in the absence of visual damage, an assessment of dye penetration should be carried out on the control and treated tissues to determine if the TER values obtained were the result of increased skin permeability or skin corrosion (Fentem et al. 1998; Botham et al. 1995). In the latter case where the stratum corneum is disrupted, the dye sulforhodamine B (Acid Red 52; Color Index 45100; CASRN 3520-42-1), when applied to the skin surface rapidly penetrates and stains the underlying tissue. This particular dye is stable to a wide range of chemicals and is not affected by the extraction procedure described below.

Sulforhodamine B Dye Application and Removal: Following TER assessment, the magnesium sulfate is discarded from the tube and the skin is carefully examined for obvious damage. If there is no obvious major damage, 150 µL of a 10% (w/v) dilution of sulforhodamine B in distilled water, is applied to the epidermal surface of each skin disc for two hours. These skin discs are then washed with tap water at up to room temperature for approximately 10 seconds to remove any excess/unbound dye. Each skin disc is carefully removed from the PTFE tube and placed in a vial (e.g., a 20 mL glass scintillation vial) containing deionized water (8 mL). The vials are agitated gently for 5 minutes to remove any additional unbound dye. This rinsing procedure is then repeated, after which the skin discs are removed and placed into vials containing 5 mL of 30% (w/v) sodium dodecyl sulfate (SDS) in distilled water and are incubated overnight at 60°C.

After incubation, each skin disc is removed and discarded and the remaining solution is centrifuged for 8 minutes at 21°C (relative centrifugal force ~175 x g). A 1 mL sample of the supernatant is diluted 1 in 5 (v/v) with 30% (w/v) SDS in distilled water. The OD of the solution is measured at 565 nm.

Calculation of Dye Content: The sulforhodamine B dye content per disc is calculated from the OD values (Fentem et al. 1998) (sulforhodamine B dye molar extinction coefficient at 565 nm = 8.7 x l04; molecular weight = 580). The dye content is determined for each skin disc by the use of an appropriate calibration curve and a mean dye content is then calculated for the replicates.

4.3.7 Interpretation of Results

The mean rat skin TER results are accepted if the concurrent positive and negative control values fall within the acceptable ranges for the testing laboratory. The acceptable resistance ranges for the rat skin TER methodology and apparatus described above are given in **Table 4-1**.

Table 4-1 Acceptable Resistance Ranges for the Rat Skin TER Methodology and Apparatus

Control	Substance	Resistance range (kΩ)
Positive	10 M Hydrochloric acid	0.5 - 1.0
Negative	Distilled water	10 - 25

The mean dye-binding results are accepted on condition that concurrent control values fall within the acceptable ranges for the method. Suggested acceptable dye content ranges for the control substances for the rat skin TER methodology and apparatus described above are provided in **Table 4-2**.

Table 4-2 Suggested Acceptable Dye Content Ranges for the Control Substances for the Rat Skin TER Methodology and Apparatus

Control	Substance	Dye content range (µg/disc)
Positive	10 M Hydrochloric acid	40 - 100
Negative	Distilled water	15 - 35

The test substance is considered to be noncorrosive to skin:

i) if the mean TER value obtained for the test substance is greater than 5 kΩ, or
ii) the mean TER value is less than or equal to 5 kΩ, and
    - the skin disc is showing no obvious damage, and
    - the mean disc dye content is well below the mean disc dye content of the 10 M HCl positive control obtained concurrently.

The test substance is considered to be corrosive to skin:

i) if the mean TER value is less than or equal to 5 kΩ and the skin disc is obviously damaged, or
ii) the mean TER value is less than or equal to 5 kΩ, and
    - the skin disc is showing no obvious damage, but
    - the mean disc dye content is greater than or equal to the mean disc dye content of the 10 M HCl positive control obtained concurrently.

4.3.8 Test Report

The test report should include the following information, if relevant to the conduct of the study:

Test and Control Substances

- Chemical name(s) such as CAS preferred name and RN, followed by other names, if known
- Purity and composition of the substance or preparation (in percentage(s) by weight)
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding)
- Stability, if known

Test Animals

- Strain and sex used
- Age of the animals when used as donor animals
- Source, housing condition, diet, etc.
- Details of the skin preparation

Justification of the Skin Model and Protocol Used

Test Method Integrity

- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time
- If the test method employs proprietary components, the procedure used to ensure their integrity from "lot-to-lot" and over time
- The procedures that the user may employ to verify the integrity of the proprietary components

Criteria for an Acceptable Test

- Acceptable concurrent negative control ranges based on historical data
- Acceptable concurrent positive control ranges based on historical data

Test Conditions

- Calibration curves for test apparatus
- Calibration curves for dye-binding test performance

- Details of the test procedure used for TER measurements
- Details of the test procedure used for the dye-binding assessment, if appropriate
- Description of any modification of the test procedures
- Description of evaluation criteria used
- Reference to historical data of the model
- Description of evaluation criteria used

Results

- Tabulation of data from the TER and dye-binding assay (if appropriate) for individual animals and individual skin samples for the test material, as well as for positive and negative controls (individual trial data and means ± S.D.), including data for replicates/repeat experiments, mean and individual values
- Description of any effects observed
- Tabulation of data from individual test samples (e.g., resistance values [kΩ] and mean dye content values [µg/disc], where appropriate)

Description of Other Effects Observed
Discussion of the Results
Conclusion

4.4 Reference Chemicals

Calibration chemicals are used to demonstrate that the validated *in vitro* rat skin TER test method is performing as expected; reference chemicals are used to determine if the performance of a new or modified *in vitro* skin TER test for skin corrosion is comparable to that of the validated reference test method. The 24 reference chemicals (12 noncorrosives, 12 corrosives) listed in **Table 4-3** provide a representative distribution of the 60 chemicals used in the ECVAM validation study of the rat skin TER assay (Fentem et al. 1998; Barratt et al. 1998) and the range of corrosivity responses obtained for the *in vivo* rabbit skin reference test method. These reference chemicals are the minimum number that should be used to evaluate the performance of a mechanistically and functionally similar, proposed test method. These chemicals should not be used to develop the prediction model for a proposed test method. If any of the recommended chemicals are unavailable, other chemicals for which adequate reference data are available could be substituted. To the extent possible, the substituted chemical(s) should be of the same chemical class as the original chemical(s). If desired, additional chemicals representing other chemical or product classes and for which adequate reference data are available can be used to more comprehensively evaluate the accuracy of a proposed test method. However, these additional chemicals should not include any that had been used to develop the prediction model for the proposed test method.

Included in this list are five organic bases, four organic acids, four inorganic acids, three electrophiles, three neutral organics, two inorganic bases, two phenols, and one surfactant. A subset of the 24 reference chemicals (12 total; 6 noncorrosives, 6 corrosives) serve as calibration chemicals for the rat skin TER assay; the names of these chemical are bolded in **Table 4-3**.

Table 4-3 Recommended Chemicals for Validation of New *In Vitro* TER Corrosivity Test Methods

Chemical[1]	CASRN	Chemical Class[2]	UN *In Vivo* PG	pH[3]
***In Vivo* Corrosives**				
Phosphorus tribromide	7789-60-8	inorganic acid	I	1.0
Sulfuric acid (10%)	**7664-93-9**	**inorganic acid**	**II/III**	**1.2**
Boron trifluoride dehydrate	13319-75-0	inorganic acid	I	1.5
Glycol bromoacetate (85%)	3785-34-0	electrophile	II/III	2.0
Caprylic acid	**124-07-02**	**organic acid**	**II/III**	**3.6**
2-tert-Butylphenol	**88-18-6**	**phenol**	**II/III**	**3.9**
60/40 Caprylic/decanoic acids	68937-75-7	organic acid	II/III	3.9
Dimethyldipropylenetriamine	**10563-29-8**	**inorganic base**	**I**	**8.3**
Dimethylisopropylamine	996-35-0	organic base	II/III	8.3
1,2-Diaminopropane	**78-90-0**	**organic base**	**I**	**8.3**
n-Heptylamine	111-68-2	organic base	II/III	8.4
Potassium hydroxide (10% aq.)	**1310-58-3**	**inorganic base**	**II**	**13.1**
***In Vivo* Noncorrosives**				
Sulfamic acid	5329-14-6	inorganic acid	NC	1.5
Isostearic acid	**30399-84-9**	**organic acid**	**NC**	**3.6**
Phenethyl bromide	**103-63-9**	**electrophile**	**NC**	**3.6**
Eugenol	**97-53-0**	**phenol**	**NC**	**3.7**
1,9-Decadiene	1647-16-1	neutral organic	NC	3.9
Benzyl acetone	2550-26-7	neutral organic	NC	3.9
Sodium lauryl sulfate (20% aq.)	151-21-3	surfactant	NC	3.9
Tetrachloroethylene	**127-18-4**	**neutral organic**	**NC**	**4.5**
4-Amino-1,2,4-triazole	**584-13-4**	**organic base**	**NC**	**5.5**
4-(methylthio)-Benzaldehyde	**3446-89-7**	**electrophile**	**NC**	**6.8**
Sodium carbonate (50% aq.)	7664-93-9	inorganic base	NC	11.7
Dodecanoic acid (lauric acid)	143-07-7	organic acid	NC	ND

Abbreviations: aq = aqueous; CASRN = Chemical Abstracts Service Registry Number; PG = Packing Group; NC = Noncorrosive; ND = not determined (unable to measure); UN = United Nations. Recommended calibration chemicals are indicated in bold type.

[1]These chemicals, sorted first by corrosives versus noncorrosives and then by pH, were selected from among the 60 chemicals used by ECVAM to validate TER (Fentem et al. 1998; Barratt et al. 1998). Unless otherwise indicated, the chemicals were tested at the purity level obtained when purchased from a commercial source (Barratt et al. 1998). The goal of the selection process was to include, to the extent possible, chemicals that: were representative of the range of corrosivity responses (e.g., noncorrosives; weak to strong corrosives) that the validated reference test method is capable of measuring or predicting; were representative of the chemical classes used during the validation process; reflected the overall performance characteristics of the validated reference test method; have chemical structures that were well-defined; induced reproducible results in the validated reference test method; induced definitive results in the *in vivo* reference test; were commercially available; and were not associated with prohibitive disposal costs.

[2]Chemical class assigned by Barratt et al. (1998).

[3]The pH values were obtained from Fentem et al. (1998) and Barratt et al. (1998).

These 12 calibration and the 24 reference chemicals are the minimum number that should be used to calibrate the validated reference test method or to evaluate the performance of a new or modified *in vitro* skin TER test for skin corrosion, respectively. While not sufficient to allow for an assessment of the ability of an *in vitro* skin TER test to accurately predict the UN Packing Group classification for a test chemical, these chemicals are adequate to assess if a rat skin TER test is functioning appropriately and to assess the extent that a modified or new skin TER test can correctly identify corrosive and noncorrosive substances. These chemicals should not be used to develop the prediction model for an alternative skin TER test method. If any of the recommended chemicals are unavailable, other chemicals for which adequate reference data are available could be substituted. To the extent possible, the substituted chemical(s) should be of the same chemical class as the original chemical(s). If desired, additional chemicals representing other chemical or product classes and for which adequate reference data are available can be used to more comprehensively evaluate the accuracy of an alternative skin TER test method. However, these additional chemicals should not include any that had been used to develop the prediction model for the alternative skin TER test method.

4.5 Accuracy and Reliability

When calibrating the performance of the rat skin TER test, 100% concordance is required for the 12 calibration chemicals (6 corrosive, 6 noncorrosive) listed in **Table 4-3**. With one exception, these 12 chemicals are the same as those listed in OECD Test Guideline 430 (*In vitro* skin corrosion: transcutaneous electrical resistance test [TER]) (OECD 2003b). Acrylic acid, proposed by the OECD as a severe corrosive, was not included because the comparative performance of this chemical in EPISKIN™ and the *in vivo* rabbit skin corrosivity test had not been demonstrated and thus the accuracy of the validated reference test method for this chemical was not established.

When evaluated using the minimum list of recommended reference chemicals in **Table 4-3**, the reliability and accuracy (i.e., sensitivity, specificity, false positive rates, and false negative rates) of the proposed *in vitro* skin TER assay should be at least comparable to that of the validated *in vitro* rat skin TER test method (ICCVAM 2002). Noncorrosive and corrosive chemicals, ranging in activity from strong to weak, and representing relevant chemical classes are included so that the performance of the proposed test method can be determined and compared to that of the validated reference test method. Based on experience with the validation of different *in vitro* test methods, one effective approach used to establish intra- and inter-laboratory reproducibility for a test method not previously validated is to test each of the reference chemicals three times in each of three independent laboratories.

The accuracy of the validated *in vitro* rat skin TER test method for the 24 reference chemicals, and the corresponding values obtained for the complete database considered by ICCVAM in its evaluation of this assay, are summarized in **Table 4-4**. The accuracy of the validated *in vitro* rat skin TER test method for the reference chemicals and the corresponding values obtained for the total database compiled during the ICCVAM evaluation process are not identical due to constraints associated with the chemical selection process.

The reliability of the proposed test method for the reference chemicals should be comparable to that of the validated *in vitro* rat skin TER test method. An assessment of interlaboratory

reproducibility is not essential if the test method is to be used in one laboratory only. In terms of cell viability measurements, the median coefficient of variation (CV) should not exceed 35% for studies conducted in different laboratories (ICCVAM 2002; Fentem et al. 1998). The median CV for replicate studies conducted in the same laboratory should be appreciably less than median CV for studies conducted in different laboratories.

Table 4-4 Accuracy of the Validated *In Vitro* Rat Skin TER Test for Skin Corrosion[1]

Source	# of Chemicals	# of Tests[2]	Sensitivity	Specificity	False Negative Rate	False Positive Rate
Reference Chemicals	24	144	86% (62/72)	75% (54/72)	14% (10/72)	25% (18/72)
Fentem et al (1998)	60	355	88% (140/159)	72% (142/196)	12% (19/159)	28% (54/196)

Definitions: Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test. Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test. False positive rate is defined as the proportion of all negative chemicals or chemical mixtures that are falsely identified as positive. False negative rate is defined as the proportion of all positive chemicals or chemical mixtures that are falsely identified as negative.

[1]The ability of the validated *in vitro* rat skin TER test method to correctly predict the *in vivo* rabbit skin corrosivity potential of the 24 reference chemicals and the corresponding performance characteristics obtained by Fentem et al. (1998) are not identical due to the constraints associated with selection of the reference chemicals. The goal of the selection process was to include, to the extent possible, chemicals that: were representative of the range of corrosivity responses (e.g., negative; weak to strong positive corrosives) that the validated reference test method is capable of measuring or predicting; were representative of the chemical classes used in the validation process; reflected the performance characteristics of the validated reference test method; have a chemical structure that was well-defined; induced reproducible results in the validated reference test method; induced definitive results in the *in vivo* reference test; were commercially available; and were not associated with prohibitive disposal costs.

[2]In the Fentem et al (1998) validation study, each chemical was tested twice in each of three laboratories (with five failed tests). Due to the presence of a balanced design, the performance characteristics are based on individual tests rather than individual chemicals.